



BIOFORTUNATM
SIMPLY DIAGNOSTICS

Product Information Document

Product Number:	BF-10-29
Product Family:	SSPGo TM
Product Name:	48 Rx HLA-DRB, DQB1 Combi Kit
Product Description:	47 reaction HLA-DRB1/3/4/5 & DQB1 genotyping by SSP comprising: 34 reactions for HLA-DRB1 1 reaction for HLA-DRB3 (DR52) group 2 reactions for HLA-DRB4 (DR53) group 1 reaction for HLA-DRB5 (DR51) group 1 reaction co-amplifies DRB4 & DRB5 subgroups. 8 reactions for DQB1
Product Packaging:	47 HLA-DRB1/3/4/5 & DQB1 reactions within individual foil pouch
Tests per Kit:	10
No template control:	Integral no template control (NTC)
Control amplification:	700bp DRA control
Stability:	16 Months from manufacture. See pack details for date. Store between 4-30°C Once a foil pouch is opened use within 3 hours

Product specifications

Resolution: The SSPGo HLA-DRB & DQB typing kit is an intermediate resolution typing kit that contains pairs of 5'- and 3'-primers for resolving the HLA-DRB1/3/4/5 and DQB1 alleles into groups based on the first two digits of the allele name. Typing at the two digit level is sometimes referred to as low or medium level resolution, whereas defining the majority of alleles to a four digit level is often termed intermediate level. 'High resolution' is often used to describe the unique identification of an allele. The BF-10-29 kit is Biofortuna's highest level of resolution for HLA-DRB and has higher level of resolution compared to other Biofortuna DRB-containing kits BF-10-30/33/34. The relatively common null alleles DRB4*01:03:01:02N and DRB5*01:08N are defined within this kit.

BF-10-29 is designed to identify the following groups of HLA-DRB & DQB alleles to at least 2-digit resolution: HLA-DRB1*01, 03, 04, 07, 08, 09, 10, 11, 12, 13, 14, 15 & 16. Most common alleles are amplified at least twice in this kit to minimise the risk of mistyping new alleles.

The following subtypes and serological subgroups are determined:

DR1	Subgroups DR103 & DR1 represented by DRB1*0103 and DRB1*01 groups (not DRB1*01:03)
DR3	Subgroups DR17 & DR18 represented by DRB1*03:01 and DRB1*03:02 groups respectively.
DR14	Subgroups DR14, DR1403 & DR1404 represented by DRB1*14:01/54, DRB14:03 and DRB1*14:04 groups respectively.
DR52	is defined by DRB3, but it is not subtyped and is represented by a single reaction.
DR53	is subtyped by 3 reactions which discriminate DRB4 alleles including all null alleles.
DR51	is subtyped into DRB5*01 and DRB5*02 groups in two reactions.

The HLA DQB1 group is resolved to the following 2-digit groups: HLA-DQB1*02, 03, 04, 05, & 06.
The DQB1*03 group is split into the serologically defined groups DQ7, DQ8 & DQ9 defined by the allele groups DQB1*03:01, *03:02 and *03:03 respectively.

Null alleles: The following HLA-DR alleles are uniquely identified:

DRB1*07:10N
DRB1*16:13N
DRB4*01:03:01:02N
DRB4*02:01N
DRB4*03:01N
DRB5*01:08N.

Version numbers: All Biofortuna kits have a version number. You must ensure the version number of the kit you are using matches the interpretation sheets and the version number in the software should you choose to use software. Version numbers change when there is a change in the kit that affects the results generated. This can occur (for example) if the primers in a kit change to accommodate a new allele or if an improved reaction has been created with a slightly different specificity to the one it replaced.

Version changes between kits: V1: Current version

SSPGo General Description: SSPGo kits are unique freeze-dried assays where complete hot-start PCR reactions are pre-dispensed into 0.2ml PCR tubes. Each reaction in the kit contains a freeze-dried PCR solution consisting of a specific primer mix of allele and group-specific primers, a control primer pair for amplifying a fragment of the DRA1 gene and all the PCR ingredients including Taq polymerase, buffer, dNTPs, Magnesium Chloride, dyes and loading buffer. The hot start dNTPs are provided under license from Trilink. The PCR reaction is dispensed in 10µl volumes and just requires a 10µl DNA sample to rehydrate the primers prior to PCR.

Contents: Each assay is contained within a foil pouch also containing a disposable desiccant bag. The assay plate is sealed with a foil thermally bonded sheet that should be removed prior to adding DNA. The PCR vessels should contain 10µl of dry solid in the base of each well; this is the complete freeze-dried PCR reaction. For orientation the first reaction is always cresol red, which appears pale pink in the dry form. The remaining wells contain a blue dye which is the same colour wet or dry.

Interpretation: Paper interpretation sheets are available from www.biofortuna.com; to aid interpretation Biofortuna have created freely available software called Verdict™ which is available through the same link. Due to the complexity of the HLA system there will be occasions when certain combinations of alleles combine to produce an ambiguous result. It is therefore recommended that the software is used to help arrive at the correct interpretation. It is further recommended that you do not use these kits as the sole method of characterising HLA for clinical decisions.

Biofortuna SSPGo kits are designed to differentiate between alleles based on the first two digits. This is sometimes referred to as 'serological level'. The relationship between serological determinants and genotyping groups can be implied by this relationship, but for more information we direct you to the subjective listing has been published by Holdsworth et al (see references).

Allele updates: All Biofortuna kits are updated on a regular basis with new alignments as they become available via IMGT HLA. Genotypes performed with kits using an earlier alignment can be retyped using updated kit information available from www.Biofortuna.com.

Primer information: The target sequence for the terminal six 3' bases of each primer are generally supplied. The forward primer information is shown as 5'-3' and the reverse primer is shown as 3'-5'. The primer location position is taken from the official alignments at <http://www.ebi.ac.uk/imgt/hla/align.html>.

No Template Control: Biofortuna's unique freeze-drying process greatly reduces the chance of PCR contamination because all you are adding is the DNA, i.e. no mixing of enzyme, buffers and DNA prior to adding to the primer mix. Therefore our single locus kits frequently do not contain a NTC well, which means our kits have improved resolution due to the extra PCR reaction. No template control reactions suitable for Biofortuna kits are available (product number BF-40-02) and can be used separately for the genotyping kit. The NTC is designed to detect possible DNA contamination (either DNA or amplicon) in the diluent used for adding the DNA.

Validation: This is a CE marked product. All Biofortuna SSPGo kits are validated against at least 48 well characterised DNA samples.

Licenses: CleanAmp™ dNTPs are licensed from Trilink Biotechnologies Inc for use in Biofortuna SSPGo products. No license to perform PCR is required to use Biofortuna SSPGo kits.

References

Bunce et al. Phototyping: comprehensive DNA typing for HLA-A, B, C, DRB1, DRB3, DRB4, DRB5 & DQB1 by PCR with 144 primer mixes utilizing sequence-specific primers (PCR-SSP). *Tissue Antigens*. 1995 Nov;46(5):355-67.

Bunce et al. Rapid HLA-DQB typing by eight polymerase chain reaction amplifications with sequence-specific primers (PCR-SSP). *Hum Immunol*.1993 Aug;37(4):201-6.

Bunce & Welsh. Molecular typing for the MHC with PCR-SSP. *Rev Immunogenet*. 1999;1(2):157-76.

Holdsworth et al. The HLA dictionary 2008: a summary of HLA-A, -B, -C, -DRB1/3/4/5, and -DQB1 alleles and their association with serologically defined HLA-A, -B, -C, -DR, and -DQ antigens. *Tissue Antigens* 73, 95–170 (2009).

Olerup & Zetterquist. HLA-DRB1*01 subtyping by allele-specific PCR amplification: a sensitive, specific and rapid technique. *H. Tissue Antigens*. 1991 May;37(5):197-204.

Revision history

This document is version 1. Dated 1-June-11